BARGER

A Study of Factors
Controlling the Rate of Regeneration

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A STUDY OF FACTORS CONTROLLING THE RATE OF REGENERATION

BY

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Panzy L. Barger

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Recommendation concurred in:

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FACTORS CONTROLLING THE RATE OF REGENERATION[1]

by

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I. INTRODUCTION

The present paper contains the results of a study of factors which influence the rate of regeneration. The factors studied are: the size of the piece, level of the cut, age, sex and quantity of food. It was found in the hydra, that a piece as small as a fourth would regenerate into a new polyp in the same length of time as a half, when both were taken from the anterior part of the hydra. The data for the level of the cut show, that within the anterior half, the level had no influence on the rate. A fourth, with its posterior cut surface at the middle, regenerated into a new individual as quickly as a fourth taken at the extreme oral end. In the case of the regeneration of the antennae of Mancasellus macrourus the rate was influenced by the level, the more proximal the level the greater the rate. In the same animals the rate was found to decrease with age, while sex and quantity of food had no influence upon it.

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[1] The research work was done at the University of Illinois, 1911-1912, under the direction of Dr. Charles Zeleny, who proposed the problems and offered many helpful suggestions for which the writer wishes to express her thanks.

II. MATERIAL

Two kinds of material were used in the experimental work; a brown hydra, probably Hydra polypus*, and a fresh-water Isopod, Mancasellus macrourus. The former furnished material for determining the effect of the size of the piece and the level of the cut. They were chosen for two reasons; first, they were easily obtained in great numbers from lily pads and fallen leaves in a pond and a small stream near the University; second, the simplicity of their structure made them capable of enduring a great degree of injury.

Mancasellus macrourus was especially suitable for such factors as level of the cut, age, sex and quantity of food. Their molting period is comparatively short and they therefore gave quick results. They also were found in great numbers near the University.

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* According to Brauer, 1909, fusca is a name that is used interchangeably for two species of brown hydra, Hydra oligactus and Hydra polypus.

Hydra oligactus is stalked; has very long tentacles, often several inches in length, when fully expanded; has three kinds of nematocysts; the embryonic chitinous membrane is spherical; has very short spines; ordinarily the sexes are separate; sexual activity is most frequent in winter; the color of the polyp is gray, brown or reddish.

Hydra polypus is stalked; the tentacles are somewhat shorter than in Hydra oligactus; it has four kinds of nematocysts; the embryonic chitinous membrane is plano-convex, with spines only on the convex side; sexual activity is more frequent in autumn; the color is gray or brown.

None of the polyps used in the above experiments were found with sex cells. In all other characteristics they agreed with the Hydra polypus.

III. EFFECT OF THE SIZE OF THE PIECE AND THE LEVEL OF THE CUT UPON THE RATE OF REGENERATION IN HYDRA.

The level of the cut and the size of the piece, although entirely different factors are so closely associated that the same experiments served to determine the effect of both.

1. Method. A number of polyps were selected and the tentacles removed just below their attachment. The body of each individual was cut into two at the middle. The anterior halves were the only parts utilized. Half of these were again cut into two equal parts. This gave three sets of pieces, viz., (1) halves with posterior level at the middle and anterior level just below the tentacles; (2) fourths with an anterior level the same as the anterior level for the halves; (3) fourths with a posterior level at the middle, the same as the posterior level of the halves.

Observations were made several times each day and a record kept of the first appearance of regeneration, which usually showed itself in several small bud-like processes, which were the beginnings of tentacles.

Sources of Error. a. Physiological Changes Due to Subjection to Laboratory Conditions. All comparisons of the rate of regeneration were made from individuals brought to the laboratory at the same time.

b. Age. This factor was eliminated on the supposition that individuals of the same size were of the same age. Individuals of as nearly the same size as possible were selected. This was done when the polyps were expanded in an aquarium jar where the environmental conditions were presumably the same and the hydras in the same state of relaxation.

c. Temperature. All regenerating pieces were kept in the same sized dishes. These were filled with water to an equal level. When the water was changed, the change was made for all at the same time. By such precautions any irregularities of temperature, which might arise from an unequal amount or a replacement of water, were prevented. The dishes were placed in a row along the window sill where the temperature conditions were believed to be uniform.

d. Differences in the Level of the Cut. All cut surfaces were made as nearly at the same level as possible. Hydra polypus is stalked and in contracting its cylindrical form is lost. The oral part assumes a more or less spherical shape while the stalk takes on the form of a truncated cone. In such cases the necessary precaution was taken to wait until the polyp had relaxed sufficiently to become cylindrical. When there was any great difference in the levels and the sizes of the pieces, they were discarded.

e. Food. The hydras regenerated within a few days, therefore no food was given during the period of regeneration.

2. Data. The pieces were divided into three series as follows:

TABLE I.

RECORD OF SERIES

Series	Parts Compared	Average Time in Hrs. for First Regeneration
A	Anterior half	51.9
B	First Fourth or Anterior Fourth	52.5
C	Second Fourth	52.8

The results for each series are given in the following tables:

TABLE II

SERIES A

Lot	Number of Pieces	Rate of Regeneration in Hrs.
I	1	89 *
"	2	65
"	2	75
"	1	87.5
II	4	60
"	2	28
"	1	52
III	3	45
"	4	48
IV	16	48
"	2	24

* Formed a bud and bud developed tentacles in 77 hrs. This seems to have delayed the formation of tentacles on the parent polyp.

TABLE III

SERIES B

Lot	Number of Pieces	Rate of Regeneration in Hrs.
I	1	77
I	2	87.5
II	1	28
"	2	52
"	3	66
"	2	112 *
III	3	45
"	3	48
"	1	56
"	1	68
IV	5	24
"	15	48

* These two pieces were considerably mashed in cutting.

TABLE IV

SERIES C

Lot	Number of Pieces	Rate of Regeneration in Hrs.
I	1	87.5
II	7	52
"	1	66
III	2	45
"	2	48
"	2	56
"	2	68
IV	11	48

A comparison of the tables for the three series shows a great variation between the four lots. It is to be noted that this is not confined to one series but is correspondingly as great for all. Therefore the general results of the experiment are not affected by it.

In Lot I, Series A, the rate varies from 65 to 89 hrs.; in B from 77 to 87.5 hrs. In C there is only one piece that lived until the end of the experiment and it formed tentacles by the end of 87.5 hrs.

In Lot II, A, the variation is from 28 to 66 hrs. (Excepting the two pieces which have already been accounted for), the limits for B are the same.

While none of C have tentacles before 52 hrs., they all have them by the end of 66 hrs.

Lot III is the only one in which the rate for the quarters is much slower than for the halves, an occurrence, which takes place in both B and C. Nevertheless five-eighths of the quarters regenerate as quickly as the halves.

In Lot IV all regenerate in 48 hrs.

The average rate is practically the same for all three series, being 51.9 for halves and 52.5 for the fourths.

If any difference in the rate whatever is indicated by the data, it is in the direction of a retardation in the beginning of the process of regeneration for the smaller pieces. However the time for the completion of the process is practically the same for all.

General Results. The general results show that a piece of Hydra polypus as small as one-fourth of the whole will regenerate into a new individual as quickly as a half, when both pieces are taken from the anterior half of the hydra. The level within the anterior half has no influence on the rate. A fourth with its anterior level just below the tentacles regenerates within the same space of time as a fourth with its posterior cut surface at the middle of the polyp.

No further work was done with the hydra. A method could not be found by which the polyps could be made to either relax or contract equally and retain that state for a time sufficient for the desired cuts to be made. Touch stimuli were tried for producing contraction. Owing to the impossiblity of applying these equally to each individual, the responses varied considerably.

Measurements were taken for the original volumes, cuts were made and the volumes of the pieces computed. As variations in contractibility produced

variations in volumes, accurate measurements and computations could not be made.

Such levels as those, immediately below the tentacles, at the middle of the polyp and fourths, made by the cutting of halves into two, could be obtained in spite of the above difficulties. It is absolutely certain that the parts compared were of two different sizes. Although they were probably not absolute halves and fourths, they were approximately such. That the levels also were different is without doubt since the two pieces compared were taken from the same individual and made by cutting the oral half of the hydra into two presumably equal parts.

3. General Discussion. a. Relation of the Size of Piece to the Rate. The determination of the smallest piece of an organism, capable of regeneration, is a problem on which considerable research has been made. Until quite recently, however, very little has been accomplished in the way of comparison of the rate of regeneration between pieces of different sizes.

Lillie (1895) found that the smallest nucleated portion of a Stentor, that would completely regenerate was a sphere with a diameter of 80 μ, or approximately 1/27 of the volume of the whole animal. In the report of these experiments he gives nothing definite as to the rates of regeneration of the different sized parts. He makes several statements, nevertheless, which seem to indicate that the larger the piece the more quickly it rearranged itself into a new individual.

Peebles (1897) places the limit of divisibility of Hydra viridis at a little less than 1/100 of the volume of the whole. The smallest piece which would form a hydra was a sphere with a diameter of 1/6 mm. She compared pieces of different sizes from the same body region and found that the larger pieces produced tentacles sooner than the smaller ones. Spheres with a diameter of

1/5-1/3 mm. regenerated about two days sooner than those 1/6 mm. in diameter.

Randolph (1897) in drawing conclusions from her observations and experiments says, "The Planaria maculata regenerates rapidly, without regard to the size of the piece or, the pharynx excepted, to the part from which it is taken".

Morgan (1898) found that almost every part of the Planaria maculata formed a new worm, the size, below which the pieces died, being less than 1/100 of the whole. He gives nothing on the relative relation of the rates of regeneration for the various pieces upon which he experimented.

Morgulis (1909) performed a number of experiments on Lumbriculus. His object was to find the relation between the initial size of the piece and the rate of regeneration. He compared halves and fourths, so cut that their posterior surfaces were at the middle of the worm. The average number of segments in the pieces were respectively, 31.6 and 16.5, practically a ratio of 2:1. The corresponding numbers of regenerated segments were 32.2 and 29, these presenting the ratio of 1.1:1. Two years later (1911) he extended his work on the same species of animal. That time he utilized halves, fourths, eighths and sixteenths, all with their posterior cut surfaces at the same level. The average number of old segments in the pieces were respectively 42.5, 26.2, 14.2 and 7.6; the number of segments regenerated, 54, 43.8, 41 and 27. The number of new segments formed per old one, within the same time was, 1.271; 1,672; 2.906 and 3.573. From that he concluded that the rate of regeneration in the small pieces was greater than in the large.

The object of the present work on hydra was to determine, if possible, if any definite relation exists between the rate of regeneration for a small and a large piece, taken from corresponding regions of similar polyps.

The results obtained show that a piece as small as one-fourth of the individual, if taken from the anterior half will regenerate new tentacles in the same time as the oral half. This, however, does not agree with the results of Peebles for Hydra viridis.

b. Relation of the Level of the Cut to the Rate. The level as a factor in regeneration dates back to the time of Spallanzani (1768) who laid the foundation of the well established fact, that the more proximal the level of remaval the greater the rate of regeneration. He observed that if the entire leg on one side and only the fingers on the opposite side were removed, from a salamander at the same time, just as much time would be required for the fingers to regenerate, as the entire leg.

Peebles (1897) while studying regeneration in Hydra viridis found that, if the ring of tentacles was removed and the body divided transversely into two or more parts, those parts from the anterior end developed a hypostome and tentacles sooner than the more posterior pieces. The small fragments from the differant body regions showed this gradation only slightly, the difference being most marked in the foot region, while the budding and reproductive zones showed no difference.

King (1898) observed in Asterias vulgaris that the arms did not regenerate equally well when cut off at different levels. The rate was greatest from the disc and decreased diredtly toward the tip of the arm.

Morgan's (1901-02-06) experiments on such animals as salamanders, earthworms, and fishes give evidence that in the same animal the regeneration of a new part proceeds at different rates, depending upon the localization of the cut. Morgulis' results (1907-09-11) are similar for Lumbricus and Podarke obscura. He says, " The rate of regeneration from different levels appears at

the very start of the regenerative process, the period of time during which there is as yet no regeneration taking place, being longer in the case of worms cut near the posterior end than those cut nearer the anterior; moreover the number of segments regenerating during any given period remains smaller in the former case than in the latter throughout the entire process of regeneration".

Ellis (1909) worked for the proportional relation between the length of the part of the tail removed, from the tadpole Rana clamitans, and that regenerated. His results show that the amount regenerated was the same whether the pieces were large or small, also that the rate increased directly with the level of the cut, as the level moved in an anterior direction.

According to Zuelzer (1907) if both antennae of Ascellus aquaticus are removed, simultaneously, but at different levels, there is a tendency for the shorter to first attain the length of the longer by more rapid regeneration then for both to continue to regenerate to their normal lengths at the same rate.

The results obtained by the writer for the level in the study of hydras were incidental. In taking different sized pieces from corresponding regions it, of necessity, involved this factor. Nevertheless, it was of interest to find that within the anterior half of the hydras tha level had no influence on the rate. The fourths from the oral end and those with posterior surface at the middle of polyp rearranged themselves into new individuals equally soon. This agreed very well with the results of Peebles for Hydra viridis, in which she found no difference in the rate for pieces from the reproductive and budding zones.

Owing to the simplicity of the structure of the polyp, one would not expect as marked a gradation as in highly differentiated tissues like the limbs of salamanders, tails of tadpoles and Oligochaets.

IV. EFFECT OF AGE, SEX, QUANTITY OF FOOD AND LEVEL OF CUT UPON THE RATE OF REGENERATION IN MANCASELLUS MACROURUS.

A. General Method. The operations consisted of the removal of antennae. In the experiments for age, sex and quantity of food, one antenna only was removed, and this at the sedond segment from the base. In the tests for level some had both antennae removed, others only one.

Sources of Error. 1, Age. This factor was controlled by selecting individuals of the same size. All Isopods used, excepting those for age, were from 5-7 mm. in length.

2. Physiological Changes Due to Subjection to Laboratory Conditions. All individuals used for comparison were brought into the laboratory at the same time.

3. Physiological Changes Due to the Molting Process. The operation, except in the experiment for age, were made at a definite period after the molt. Also the measurements of the parts regenerated were taken with special reference to the preceding molt, the time at which the measurements were made corresponding to that between the first molt and the operation.

4. Differences in the Level of the Cut. All cuts were made at the articulation of segments where the exact level was easy to determine.

5. Additional Injury. All individuals with the additional loss of any of the appendages were discarded.

6. Temperature. The laboratory was not kept at a constant temperature, but the regenerating Isopods were placed in the same sized dishes, which were arranged side by side on a table where all were subjected to the same variations.

7. Food. The food consisted of water-soaked elm leaves, which were placed in the dishes in about equal quantities, at the beginning of the experiment.

B. Effect of Age upon the Rate

1. Method. Thirty-four females, 6 mm. and thirty-six 18 mm. in length were selected. There was enough difference in the size of these two lots so that there could be no doubt that they were of different age. So much difference would not be due to food relations. One antenna was removed from each, soon after they were brought to the laboratory, without waiting for them to molt. The experiment was continued through a period of 21 days.

2. Data. Tables V and VI give the results of the experiment.

TABLE V.

(individuals 18 mm. in length)

No.	First Molt	Length Reg.	Second Molt	Length Reg.	Remarks
1	11	8			
1	13	9			
1	13	12			
1	16	12			
1	9	18			
1	17	11			
1	20	17			
9					Molted but no regeneration
20					Died before molt

The first column gives the number of individuals.

The second column gives the number of days between operation and the first molt.

The third column gives the length regenerated in segments.

The fourth column gives the number of days between the operation and the second molt.

The fifth column gives the length regenerated between the operation and second molt.

TABLE VI

(individuals 6 mm. in length)

No.	First Molt	Length Reg.	Second Molt	Length Reg.	Remarks
1	8	14	15	29	
1	8	9			Died
1	8	10			Died
1	8	10	20	24	
1	8	16	15	28	
1	8	16	17	30	
1	8	11	15	23	
1	8	14			Died
1	8	11	19	25	
1	9				Both antennae lost in the molt
1	9	12	20	26	
1	9	20	17	32	
1	10	15			
1	10	16	18	32	
1	10	18	19	32	
1	10	18			
1	10	18			
1	12	19			Died
1	12	23	18	33	
1	14	21			
1	14	25			
1	14	28			
1	14	16			

TABLE VI, continued

(individuals 6 mm. in length)

No.	First Molt	Length Reg.	Second Molt	Length Reg.	Remarks
1	14	26			
1	14	21			
1	14				Both antennae lost in the molt
1	15	25			
1	16	26			
1	17	22			
1	17	30			
4					Died before the molt

For explanation of table see page 16.

At the end of the experiment, sixteen (44 per cent) of the older ones were alive and of these eleven (31 per cent) had regenerated new segments. Thirty (89 per cent) of the younger ones were alive and all of these (100 per cent) had molted once and had new segments, while eleven (37 per cent) had molted twice.

The number of segments regenerated was taken as the basis for comparison. Although the segments differ in size for the two lots, the segments of each are in relative proportion to the size of the individual.

With one exception[1] the older ones showed no signs of regeneration for 11, 13, 16, 17 and 20 days. Then the average number of segments regenerated was respectively, 8, 10.5, 12, 11 and 17. The average number of segments regenerated by the younger in 10 days was 17; in 12 days, 21; in 16 days, 26, and in 17 days, 28.5. The number regenerated by the older ones is approximately one half that regenerated by the younger in the same period of time.

General Results. Under laboratory conditions the death rate is much greater for the old than for the young, the period between molts is irregular for all, but is longer for the old, as is also the rate of regeneration.*

3. Discussion. It is generally known that, as a rule, the rate of regeneration decreases with an increase in age. As early as 1768 Spallanzani observed this in regenerating tadpoles. Ellis (1909) verified it in tadpoles of Rana clamitans. In the same year, Zeleny (1909) experimented upon eight diffêrent species of animals, viz., Cassiopea Xamachana, Ophioglypha lacertosa, Portunus sayi, Cambarus propinquus, Cambarus bartoni, Palaemonetes vulgaris, Amblystoma jeffersonianum and Palaemon tenuicornis. His general conclusion, for all except Portunus and the younger individuals of Ophioglypha, was that "In older individuals it takes longer to complete a removed organ than in younger ones". Portunus sayi and the young Ophioglypha acted in an opposite

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[1] This one regenerated eighteen segments in 9 days. The increase may have been due to successive removal. This is a statement for which I have no direct evidence, as the earlier history of the animal was not known. Such errors could only be eliminated by keeping a rêcord of each individual from the time it leaves the brood pouch until it has grown sufficiently for operation.

* There is a possibility of error here, due to the disregard of the molting period, with respect to the operation.

way. The former with an increase in size showed an increase in rate. In the latter there appeared three periods in which the rate differed. During two of these, when old and also very young, the rate was slow, while during the intermediate stage regeneration took place more rapidly. Only two sizes of Mancasellus were used for the present data. The results from these agree with the majority of cases, a decrease of rate with an increase of age.

C. Influence of Sex upon Regeneration

1. Method. Twenty individuals 6 1/2 to 7 mm. in length were used for this experiment. One antenna of each was removed at the second segment, on the day following the molt[1]. On the day following the second molt the lengths of the regenerated antennae were measured.

2. Data. Column 7 in Table VII and VIII gives the lengths in mm. regenerated for each antenna, between the operation and the following molt. Column 9 gives the number of days between the operation and the following molt. The latter is seen to be very irregular. Owing to this it is impossible to compare directly the lengths regenerated between each molt. It necessitates a reduction to the length regenerated per day. For the males, this length is fairly constant, the average being .26 mm. The females show a greater variation and the average is .28 mm., slightly greater than that for the males. Whether this difference is due to the difference in the number of animals compared or to sex cannot be determined definitely from the given data.

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[1] Only one half of Mancasellus molts at a time. In every case observed, the posterior half molted first and generally, on the following day, the anterior half molted. All records were made of the anterior molts only.

TABLE VII

MALES

Catalog Number	First Molt	Operation	Length Remaining after Operation mm.	seg.	Second Molt	Length Regenerated mm.	seg.	No. Days Between Operation and * Molt	Rate per Day
1	4/21	4/22	.51	2	5/3	2.58	23	11	.23
3	4/22	4/23	.51	2	5/4	2.79	31	11	.25
5	4/22	4/23	.51	2	5/3	2.75	24	10	.27
7	4/22	4/23	.51	2	5/5	3.10	29	12	.26
12	4/23	4/24	.51	2	5/3	2.23	23	9	.25
18	4/23	4/24	.51	2	5/4	2.93	28	10	.29
42	4/24	4/25	.51	2	5/4	2.58	22	9	.29
44	4/24	4/25	.51	2	5/3	2.41	21	8	.30
47	4/25	4/26	.51	2	5/3	1.67	18	7	.24
53	4/25	4/26	.51	2	5/8	2.37	19	12	.20
Av.			.51	2		2.54		9.9	.26

* second molt

TABLE VIII

FEMALES

Catalog Number	First Molt	Operation	Length Remaining After Operation mm.	seg.	Second Molt	Length Regenerated mm.	seg.	No. Days Between Operation and* Molt	Rate per Day
2	4/21	4/22	.51	2	5/3	3.10	29	11	.28
4	4/22	4/23	.51	2	5/4	2.96	27	11	.27
6	4/22	4/23	.51	2	5/5	3.10	28	12	.26
14	4/23	4/24	.51	2	5/11	2.98	28	17	.18
16	4/23	4/24	.51	2	5/4	3.05	30	10	.30
19	4/23	4/24	.51	2	5/8	2.68	33	14	.19
20	4/23	4/24	.51	2	5/3	2.93	24	9	.32
45	4/24	4/25	.51	2	5/3	3.01	26	8	.37
52	4/25	4/26	.51	2	5/3	2.15	19	7	.30
48	4/25	4/26	.51	2	5/3	2.41	18	7	.34
Av.			.51	2		2.84		10.6	.28

* second molt

3. Discussion. Sex as a factor in regeneration has received very little attention. Zeleny (1905) found the rate of regeneration and the molting period to be different for the sexes of Cambarus propinquus.

It seems probable that it would have an influence on regeneration, especially in adults or individuals becoming sexually mature, when the physiological conditions are necessarily changing. If such be the case no difference should be expected in very young individuals which are sexually immature. In the young Mancasellus it seems safe to conclude then that sex has no influence upon the rate of regeneration.

D. Quantity of Food as Factor in Regeneration

1. Method. Both males and females, 5 to 6 mm., were used. One antenna was removed from each, at the second segment, three days after the molt. Two series were compared.

(a) Well fed

(b) Starved.

2. Data. All but one of the well fed individuals lived through the experiment and by the end of eleven days after the operation all had molted. Eight out of fourteen of those without food lived but they had not finished molting until after twenty days. The average number of days between the operation and the following molt was eight days for the former and fourteen for the latter. This difference was quite marked and indicated a delaying of the molting process by starvation.

The average length regenerated per day by those fed was .24 mm., for those starved. 19 mm. The question at once arises here, whether the difference

is due to a difference in the rate of regeneration or a retardation in the molting process of those deprived of food.

According to Ellis (1909) and Durbin (1909), regeneration takesplace slowly during the first few days after the operation, then increases rapidly for a time and finally decreases slowly to zero.

If this be true for Mancasellus it is easily possible for the molting to be delayed until after the decline in rate begins. Thus the average amount regenerated per day by the starved ones will be lowered by dividing the amount regenerated by the number of days, which not only represent the maximum period but also a part of the decline.

General Results. The molting process is delayed by starvation. The rate of regeneration if influenced is decreased only slightly, the decrease as shown by the data probably being due to the delay of the molting period over the time of maximum regeneration.

3. Discussion. The role that food plays in regeneration has not as yet become universally established. There are some cases on record in which no definite relation between food supply and the rate of regeneration could be found, for instance in planarians deprived of food, Morgan found extensive regeneration taking place. He also found that in the Salamander Diemyctylus viridiscens the rate of regeneration in the length of the tail was independent, to a great degree, of the quantity of food. The amount of new material produced by the starved individuals was less, but in order to become symmetrical, relatively less was required since the whole animal was smaller. Spallanzani observed that tadpoles not fed would cease to grow, but still would regenerate their tails if they were cut off.

That food has some influence is shown by the observations of Durbin (1909) for tad poles, Rana clamitans. Irregularities in the food supply pro-

duced corresponding irregularities in the rate of regeneration of the tail. Morgulis' (1909) conclusions for Podarke obscura were similar. Well fed worms regenerated more segments and longer tails than worms without food.

The present data show a delay in the rate of regeneration, which, however, is believed to be due to the delay of the molting process beyond the period of maximum regeneration.

TABLE IX

STARVED

Catalog Number	First Molt	Operation	Second Molt	Length Regenerated mm.	seg.	No. Days between Operation and Second Molt	Rate per Day
201	4/26	4/29	5/19	2.92	31	20	.15
204	4/28	5/1	5/19	2.24	24	18	.12
206	4/28	5/1	5/11	2.23	26	10	.22
210	4/28	5/1	5/9	2.25	24	8	.28
216	4/28	5/1	5/13	2.51	29	12	.21
220	4/28	5/1	5/17	2.79	27	16	.17
222	4/28	5/1	5/19	2.51	23	18	.14
232	5/3	5/6	5/16	2.15	27	10	.21
Av.				2.45		14	.19

TABLE X

Fed

Catalog Number	First Molt	Operation	Second Molt	Length Regenerated mm.	seg.	No. Days between Operation and Second Molt	Rate per Day
200	4/26	4/29	5/4	1.31	12	5	.26
202	4/26	4/29	5/7	2.41	16	8	.30
203	4/28	5/1	5/8	2.05	19	7	.29
205	4/28	5/1	5/13	2.48	26	12	.21
209	4/28	5/1	5/11	2.32	20	10	.23
213	4/29	5/2	5/11	1.72	14	9	.19
215	4/29	5/2	5/9	1.74	17	7	.25
217	4/29	5/2	5/13	3.36	27	11	.30
219	4/29	5/2	5/11	1.63	16	9	.18
221	4/29	5/2	5/8	1.12	11	6	.19
227	5/3	5/6	5/13	1.81	18	7	.26
229	5/3	5/6	5/17	2.32	21	11	.21
Av.				2.02		8.5	.24

TABLE XI

Right Antenna Removed at the Second Segment

Catalog Number	First Molt	Length of Right Antenna		Date of Operation	Length Removed from Right Antenna		Length Remaining on Right Antenna		Second Molt	Length Regenerated by Right Antenna		Length of Right Antenna		Length of Left Antenna	
		mm.	seg.		mm.	seg.	mm.	seg.		mm.	seg.	mm.	seg.	mm.	seg.
21	4/22	4.47	43	4/23	4.13	41	.34	2	5/1	0	0				
21	4/22	4.05	40	4/23	3.71	38	.34	2	5/2	2.93	23				
24	4/23	4.74	42	4/24	4.40	40	.34	2	*						
25	4/23	4.77	40	4/24	4.43	38	.34	2	5/3	1.93	19				
26	4/23	4.18	39	4/24	3.84	37	.34	2	5/2	2.18	19				
27	4/23	3.44	31	4/24					5/2			4.41	37	3.70	30
28	4/23	4.39	42	4/24	4.05	40	.34	2	5/1	0	0				
29	4/23	3.39	35	4/24					4/30			3.87	36	3.87	35
30	4/23	4.48	38	4/24					*						
31	4/23	2.93	30	4/24	2.59	28	.34	2	*						
32	4/23	2.93	26	4/24	2.59	24	.34	2	5/2	1.55	15				
33	4/24	3.79	33	4/25	3.45	31	.34	2	5/2	1.52	14				
34	4/24	4.31	41	4/25	3.88	39	.43	2	5/3	2.32	19				
35	4/24	4.22	42	4/25	3.88	40	.34	2	5/2	1.77	18				
36	4/24	4.65	42	4/25	4.22	40	.43	2	5/3	2.23	16				
37	4/24	3.96	37	4/25					5/1			4.82	42	3.48	37†
38	4/25	3.60	28	4/26	2.22	26	.34	2	5/3	0	0				
39	4/25	4.13	41	4/26	3.79	39	.34	2	5/3	2.06	18				
40	4/25	4.13	37	4/26	3.79	35	.34	2	5/3	1.55	16				

* Died before the second molt
† Left antenna broken at tip

TABLE XII

Left Antenna Removed at the Fourth Segment

Catalog Number	First Molt	Length of Left Antenna mm.	seg.	Date of Operation	Length Removed from Left Antenna mm.	seg.	Length Remaining on Left Antenna mm.	seg.	Second Molt	Length Regenerated by Left Antenna mm.	seg.	Length of Right Antenna mm.	seg.	Length of Left Antenna mm.	seg.
21	4/22	3.44	33	4/23	2.24	29	1.20	4	5/1	.96	12				
22	4/22	3.36	26	4/23	2.16	22	1.20	4	5/2	.94	11				
24	4/23	4.31	38	4/24	3.11	34	1.20	4	*						
25	4/23	4.48	36	4/24	3.28	36	1.20	4	5/3	1.15	13				
26	4/23	4.31	41	4/24	3.11	37	.34	2°	5/2	2.15	19				
28	4/23	4.05	37	4/24	2.76	33	1.29	4	5/1	.48	5				
30	4/23	4.48	42	4/24					*						
31	4/23	4.39	42	4/24	3.19	38	1.20	4	*						
32	4/23	3.79	35	4/24	2.59	31	1.20	4	5/2	.60	8				
33	4/24	3.96	36	4/25	2.59	32	1.37	4	5/2	.48	6				
34	4/24	4.34	38	4/25	3.05	34	1.29	4	5/3	1.03	11				
35	4/24	4.70	43	4/25	3.33	39	1.37	4	5/2	.55	8				
36	4/24	4.39	42	4/25	3.19	38	1.20	4	5/3	1.03	9				
38	4/25	4.13	34	4/25	2.93	30	1.20	4	5/3	.51	5				
39	4/25	3.65	33	4/26	2.45	29	.34	2°	5/3	2.06	19				
40	4/25	4.13	38	4/26	2.93	34	1.20	4	5/3	1.10	9				
41	4/25	4.50	39	4/26					5/4			4.17	32	3.63	28+
42	4/25	4.31	38	4/26					5/2			4.92	41	4.82	4[illegible]
43	4/25	3.62	37	4/26					*						

° Left antenna was thrown off at second segment, after the operation
*Died before molting
+Antenna broken at the tip

TABLE XIII

Catalog Number	Length of Right Antenna mm.	Length Removed mm.	Part of Whole Removed	Length of Left Antenna mm.	Length Removed mm.	Part of Whole Removed
22	4.05	3.71	.92	3.36	2.16	.64
25	4.77	4.43	.93	4.48	3.28	.73
32	2.93	2.59	.88	3.79	2.59	.68
33	3.79	3.45	.91	3.96	2.59	.66
34	4.31	3.88	.90	4.34	3.05	.70
35	4.22	3.88	.92	4.70	3.33	.71
36	4.65	4.22	.91	4.39	3.19	.73
40	4.13	3.79	.92	4.13	2.93	.71
Av.	4.10	3.74	.91	4.14	2.39	.69

E. Influence of the Level of the Cut upon the Rate

1. Method. The rate of regeneration of a single antenna was determined in each of the following cases:

a- One antenna removed at second segment plus one removed at the fourth

b- One antenna removed at fourth segment plus one removed at the second

c- One antenna removed at second segment

d- One antenna removed at fourth segment

2. Data. The data for a and b, in Tables XI and XII, were obtained from the same individuals. Twenty-two Isopods were used, six of these as control individuals. The antennae of the remaining were removed, the right at the sedond segment and the left at the fourth. Table XIII (derived from

XI and XII) gives the average length removed from the right antenna as (.91) approximately nine-tenths of the whole, and the left (.69) approximately seven-tenths.

Owing to factors which could not be controlled only eight individuals of the series were available for comparison.*

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* No. 26 and 39 (Table XII) threw off the left antenna shortly after the operation, thus making the level of the cut surface of both antennae the same. After the second molt, No. 21, 28 and 38 showed no new segments on the right antenna but had regenerated the following lengths on the left, .96 mm., .48 mm. and .51 mm. Two, explanations may be offered:

(1) The absence of new segments at the proximal level might have been due to a loss of such at the molt. It is a very common occurrence for the normal individuals to lose one or even both antennae during this process. Another observation that might lead to the belief that new segments were formed but had been broken off was the smoothness of the surface at the end of the right antenna. If such were not the case one would have expected the surface to be slightly rounded, at least.

(2) The more plausible explanation is that the right one, owing to the increased length of removal, had not yet started to regenerate. Ellis (1909) says, "The first slow period of regeneration increases in length the higher the level of injury".

TABLE XIV

Catalog Number	First Molt	Operation	Second Molt	Length Regenerated by Right Antenna	Length Regenerated by Left Antenna	No. Days between Operation and Second Molt	Length Regenerated per Day by Right	Length Regenerated per Day by Left
22	4/22	4/23	5/2	2.93	.94	9	.32	.10
25	4/23	4/24	5/3	1.93	1.15	9	.21	.13
32	4/23	4/24	5/2	1.55	.60	8	.19	.07
33	4/24	4/25	5/2	1.52	.48	7	.22	.07
34	4/24	4/25	5/3	2.32	1.03	8	.29	.13
35	4/24	4/25	5/2	1.77	.55	7	.25	.08
36	4/24	4/25	5/3	2.23	1.03	8	.28	.13
40	4/25	4/26	5/3	1.55	1.10	7	.22	.16
AV				1.97	.86	8	.25	.11

TABLE XV

Catalog Number	First Molt	Operation	Second Molt	Length Regenerated mm.	No. Days between Operation and Second Molt	Rate per Day mm.
90	4/27	4/28	5/5	2.06	7	.29
91	4/27	4/28	5/5	1.98	7	.28
94	4/28	4/29	5/8	2.32	9	.26
98	4/28	4/29	5/5	2.01	6	.37
100	4/28	4/29	5/8	2.05	9	.23
106	4/28	4/29	5/13	2.87	14	.20
109	4/28	4/29	5/8	2.37	9	.26
111	4/29	4/30	5/5	1.98	5	.39
114	4/29	4/30	5/5	1.24	5	.25
Av.					8	.28

In every case as Table XIV shows the length regenerated at the proximal level is greater than at the distal. The average rate per day for the former is .25 mm. and for the latter, .11 mm., presenting the ratio of 2.3 : 1. The parts removed are in the ratio of 9 : 7. The proportion between these ratios does not show a direct proportion between the length removed and the length regenerated.

TABLE XVI

Catalog Number	First Molt	Operation	Second Molt	Length Regenerated mm.	No. Days between Operation and Second Molt	Rate per Day mm.
92	4/27	4/28	5/5	.91	7	.13
115	4/29	4/30	5/5	.55	5	.11
101	4/29	4/30	5/15	1.73	15	.11
110	4/28	4/29	5/5	.72	6	.12
112	4/29	4/30	5/5	.72	5	.14
Av.					8	.12

c. One antenna removed at the second segment. The results for this experiment are recorded in Table XV. The average rate of regeneration per day is .28 mm., practically the same as that for the proximal level in a.

d. One antenna removed at the fourth segment, Table XVI. The rate of regeneration per day at the distal level is .12, practically the same as in experiment b.

A comparison of Tables XV and XVI verifies the results obtained in experiments a and b, viz., the rate at the more proximal level is greater than that at the more distal one. The ratio between the lengths regenerated per day in c and d is 2.31 : 1, the same as in a and b.

General Results. The rate of regeneration is greater at a proximal than at a distal level. The length regenerated is not in direct proportion

to the length removed (lengths removed are as 9 : 7). Instead the ratio is as 2.3 : 1. There is no visible increase in rate with the additional removal of a second antenna.

3. Discussion. The present status of the effect of level has already been discussed in Part I of this paper. It is of interest to note here the peculiar proportion between the ratio of the parts removed and the ratio of the parts regenerated. The growth from the distal level seems to lag behind. There was a difference in the cut surfaces which might have given rise to this variation. The antenna is composed of four broad segments and a large number of small ones. At the place of articulation of the small with the broad, a great part of the distal end of the latter is a free surface. In the antenna cut at the second segment a large cut surface was exposed while in those cut at the fourth, only about one-third as much surface was exposed. Whether this difference in exposed surfaces influenced the rate is not known. This could have been tested by making the cut immediately on the proximal side of the articulating surface, but time did not permit.

V. SUMMARY.

1. A piece of Hydra polypus as small as a fourth will regenerate into a new polyp as quickly as a half, if both are taken from the anterior part of the hydra.

2. The level within the anterior half of Hydra polypus has no influence upon the rate of regeneration.

3. In Mancasellus macrourus the rate of regeneration decreases with an increase in age.

4. Sex has no influence upon the rate.

5. Starvation increases the length of the periods between molts.

6. A slight decrease in the rate of regeneration appears among starved animals, due either to starvation directly or a delay in the process of molting until after the maximum rate of growth has passed.

7. The more proximal the level the greater the rate of regeneration.

8. The regeneration ratio, when the antennae are removed at the second and fourth segments respectively, is as 2.3 : 1.

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